Inoculation of Nursery Seedbeds With Pisolithus Tinctorius Spores Mixed With Hydromulch Increases Ectomycorrhizae and Growth of Loblolly Pines

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ABSTRACT. Different methods of introducing basidiospores (4/5 oz. spores/100 linear ft. of nursery bed) of Pisolithus tinctorius *into fumigated soil at Weyerhaeuser's nursery in Oklahoma were tested to determine their effectiveness in forming ectomycorrhizae on loblolly pine seedlings. Two of five methods proved significantly effective. Nearly three-fourths of seedlings treated with spores mixed in hydromulch and applied after sowing formed* Pisolithus *ectomycorrhizae. The result was 25 percent larger seedlings and 15 percent fewer culls. In plots where spores were dusted onto the soil at sowing, one-third of the seedlings formed* Pisolithus *ectomycorrhizae, resulting in 12 percent larger seedlings and 13 percent fewer culls.*

Considerable research has been conducted recently on the use of pure cultures of fungi to increase ectomycorrhizal development and growth of forest tree seedlings. In greenhouse studies in Australia, Theodorou (1971) and Theodorou and Bowen (1973) found that coating seeds of *Pinus radiata* with basidiospores of *Rhizopogon luteolus* is an easy and effective method to introduce ectomycorrhizal fungi into potting soil. In Australia, Lamb and Richards (1947a, b, c) used basidiospores of *R. roseolus*, *Suillus granulatus*, and *Pisolithus tinctorius* to increase ectomycorrhizal development and growth of *P. radiata* seedlings in natural soils deficient in fungal symbionts.

In this country, *P. tinctorius* has been successfully introduced into soils of nurseries in Florida and North Carolina (Marx and others 1976), Oklahoma (Marx and others 1978), Virginia (Marx and Artman 1978), and other southern states (unpublished data). In most cases the ectomycorrhizae formed with *P. tinctorius* significantly increased seedling growth of several species of pine. When pine seedlings with abundant *Pisolithus* ectomycorrhizae were planted on southern reclamation (Marx 1977) and routine reforestation sites (Marx *et al.* 1977), they survived and grew better than routine nursery seedlings having naturally occurring ectomycorrhizae. In the nursery tests, vegetative mycelium grown in pure culture in the laboratory in vermiculite-peat moss-nutrient medium was found to be consistently better than basidiospores for synthesizing ectomycorrhizae and stimulating seedling growth. The effectiveness of basidiospore inoculum was decreased in those nurseries where there was intense competition from other ectomycorrhizal fungi. With minimum competition, such as was encountered at the Oklahoma nursery, the basidiospores were very effective in forming ectomycorrhizae and stimulating seedling growth (Marx *et al.* 1978). On the basis of these results, further research on the use of basidiospores appeared justified. We therefore tested different methods of introducing basidiospores of *P. tinctorius* into fumigated soil at the Oklahoma nursery to ascertain their effect on ectomycorrhizal development and growth of loblolly pine (*Pinus taeda* L.) seedlings.

MATERIALS AND METHODS

Dry basidiospores of *P. tinctorius* were extracted (Marx 1976) from mature basidiocarps collected in September 1975 under loblolly pine growing on a kaolin spoil near Macon, Georgia. The dry basidiospores were stored in amber bottles at 5°C. The amount of basidiospores used for all treatments in this test was approximately 4/5 oz./100 linear ft. of nursery bed. The experiment was installed at Weyerhaeuser's Fort Towson, Oklahoma nursery in a section where loblolly pine seedlings grew the previous year. In early spring of 1976, the soil was fertilized with 750 lb./acre of NH_4NO_3, 500 lb./acre of 12-12-12, 750 lb./acre of dolomitic lime, and 600 lb./acre of 10-20-20. All fertilizers were broadcast and disked into the soil. The nursery section also was sprayed with Captan® (12 lb./acre) and Terrachlor® (1 pt./acre) prior to sowing. The soil was fumigated in March 1976 with methyl bromide (MBC-2) applied under clear plastic at the rate of 318 lb./acre. The plastic was removed after 3 to 5 days, and the beds were shaped. Each of the five nursery units used in this test had nine 4- by 600-ft. beds. A 100-ft.-long test plot was laid out on both ends of beds 4, 6, and 8 in each unit to provide a total of 30 plots. Each nursery unit of six test plots was a

replicate block; each block contained one plot of each of six treatments.

The following six treatments were randomly installed in the six plots of each of the five nursery units.

Treatment 1.—Basidiospores mixed in hydromulch after sowing.

Loblolly pine seeds were sown prior to installing the treatment on April 7, 1976. Approximately 4 oz. of basidiospores were mixed thoroughly in 1.5 gallons of water with 30 drops of wetting agent (Tween 20®) in a 2-gallon plastic bottle. The basidiospore suspension was poured into a conventional Bowie® hydromulcher containing 275 gallons of water, 83 lb. of hydromulch (Silvafiber®, Weyerhaeuser Company), and 13 quarts of sticker (Petroset SB®, Phillips 66). This volume, only one-third of a load for the hydromulcher, was spread evenly over the 100-ft. plots in each of the five units within 30 minutes of adding the spores to the hydromulcher.

Treatment 2.—Basidiospores dusted onto soil after sowing.

Plots were seeded and irrigated to moisten the soil just prior to treatment application on April 8, 1976. Approximately 4 oz. of basidiospores were placed in a small, hand-operated pesticide duster (Pestmaster Garden Duster®, D. B. Smith & Co., Utica, New York). Spores were dusted evenly on the moist soil of the five plots during a wind-free period. The plots were immediately irrigated and covered with hydromulch.

Treatment 3.—Basidiospores injected into soil before sowing.

Four ounces of spores were placed in a 3-gallon, pressurized paint sprayer and injected 4 inches deep into the soil through five shanks spaced 10 inches apart on a tractor-drawn rig; N_2 gas was used as the carrier. The five plots were seeded and hydromulched after the spores were injected on April 8, 1976.

Treatment 4.—Basidiospores dusted onto seedlings 6 weeks after sowing.

Plots were seeded and hydromulched on April 8, 1976. Six weeks later, on May 12, plots were irrigated to moisten the seedlings and the soil. Basidiospores were then dusted onto seedlings and the soil at the same rate and method used in Treatment 2. Immediately after dusting, the plots were irrigated to wash the spores into the root zone.

Treatment 5.—Basidiospores drenched onto seedlings 6 weeks after sowing.

In this treatment, plots were seeded and hydromulched on April 8, 1976. On May 13, basidiospores were placed in the hydromulcher containing water and sticker only (hydromulch fiber was omitted) and then drenched onto seedlings at the same rate and method used in Treatment 1.

Treatment 6.—Controls without basidiospores.

Control plots were treated as other plots, but spores were not added to the soil.

Loblolly pine seed used in this test were collected from a Mississippi-Alabama source in 1975 and treated with Latex and Arasan® (42-S). The rate of hydromulch was 1200 lb./acre with 2.4 percent Petroset SB® by volume.

During the growing season, all plots were fertilized uniformly with $(NH_4)_2SO_4$ and KCl according to the following schedule: June, 25 lb. N/acre; July, 30 lb. N/acre; August, 30 lb. N/acre; in the fall the plots were fertilized with 50 lb. K/acre to promote cold hardening of seedlings.

Seedlings were lifted and evaluated during the week of January 25, 1977. Prior to lifting, all plots were undercut with a root-pruning bar at a depth of 8 inches and laterally pruned between seedling rows. All seedlings were removed by hand from five 2- by 4-ft. subplots spaced 1, 25, 50, 75, and 99 ft. from one end of each 100-ft. test plot. These seedlings were counted and graded. Seedlings less than 6 inches tall, with stems less than 1/8 inch in stem diameter at the root collar, with forked tops, or without dormant buds were counted but discarded as culls. Ten plantable seedlings from each subplot were chosen at random for measurement of stem height, stem diameter at root collar, and fresh weights of shoots and roots. The degree of ectomycorrhizal development on each seedling was estimated visually (Marx *et al.* 1976). Data from subplots were totaled and averaged to represent the mean for the plot.

The seedlings in the 90 linear feet of nursery bed remaining per plot were machine-lifted and graded by Weyerhaeuser personnel on a conveyor line. On the conveyor line, seedlings less than 6 inches tall or taller than 14 inches and with stems less than 1/8 inch in diameter were counted as culls and discarded. Over 38,000 seedlings were removed from subplots by hand for detailed seedling evaluation, and over 310,000 seedlings were lifted by machine and graded on the conveyor line by Weyerhaeuser personnel. The data were subjected to analyses of variance. Differences among treatment means were separated with Duncan's Multiple Range Test.

Mixing basidiospores in hydromulch at sowing (Treatment 1) was the most effective method used in this study (Table 1). Nearly three-fourths of the seedlings had *Pisolithus* ectomycorrhizae, and approximately one-fourth of the total amount of ectomycorrhizae on seedlings was formed by *Pisoli-*

Table 1. Seedling and plot data from loblolly pine grown in nursery beds infested by different methods with basidiospores of the ectomycorrhizal fungus *Pisolithus tinctorius* (Pt)[1]

Basidiospore inoculation method	Seedling data[2]					Plot data[3]	
			Fresh wt. of 1,000 seedlings	Pt ectomycorrhizae			
	Height	Stem diam.		All ectomycor.	Seedlings	Total seedlings	Plantable seedlings
	Inches		*Lb.*	*Percent*		*Number*	*Percent*
Mixed in hydromulch after sowing	8.0 a	0.20 a	34.8 a	28 a	72 a	54,350 a	80.4 a
Dusted onto soil after sowing	7.6 b	0.18 b	30.8 b	10 b	36 b	50,575 b	78.7 a
Injected into soil before sowing	6.9 c	0.17 b	29.1 bc	6 c	24 c	50,405 b	74.3 ab
Dusted onto seedlings 6 weeks after sowing	7.4 ab	0.18 b	29.1 bc	6 c	26 c	52,015 ab	70.5 b
Drenched onto seedlings 6 weeks after sowing	7.2 bc	0.18 b	28.9 bc	4 c	22 c	50,650 b	69.3 b
Control (no basidiospores added)	7.0 c	0.17 b	27.5 c	0.2 d	0.01 d	52,770 ab	69.7 b

[1] *Column means followed by a common letter are not significantly different ($P = 0.05$).*
[2] *Means obtained from 10 randomly selected seedlings lifted by hand from each of five 8-sq.-ft. subplots located in each of 5 blocks per treatment.*
[3] *Seedlings machine-lifted from five 90-linear-ft. plots per treatment (those remaining after seedlings removed from the five subplots); counted and graded by Weyerhaeuser personnel on conveyor line.*

thus. These *Pisolithus* ectomycorrhizae induced a 25-percent increase in fresh weight of seedlings and increased the number of plantable seedlings by 15 percent over the controls. The next most effective treatment for forming *Pisolithus* ectomycorrhizae was dusting basidiospores onto soil at sowing (Treatment 2). Approximately one-third of the seedlings had *Pisolithus* ectomycorrhizae, and these represented 10 percent of all the ectomycorrhizae. This *Pisolithus* development, however, increased fresh weight of seedlings by 12 percent and the number of plantable seedlings by 13 percent over the controls. The remaining three treatments did not significantly affect seedling size or number of plantable seedlings. Very few seedlings in these latter treatments had *Pisolithus* ectomycorrhizae.

CONCLUSION

The mixing of basidiospores of *P. tinctorius* with hydromulch and applying them with the hydromulcher after seeding is a simple, rapid, and practical method to infest nursery soil with *P. tinctorius*. This method does not require any change in normal nursery operations, assuming that these operations include hydromulching. Initially, there was concern that the basidiospores would not leach from the wood fiber into the root zone, especially after the addition of the sticker to the wood fiber. This concern, as well as possible toxicity of the sticker to the spores, was unfounded. In a separate test (unpublished) in Athens, spores were mixed with various amounts of hydromulch and sticker for different periods of time prior to mulching soil. It was found that hydromulch and sticker had no significant effect on the function of basidiospores as inoculum for *Pisolithus* ectomycorrhizal development.

Use of basidiospores of *P. tinctorius* instead of vegetative inoculum to infest soil in nursery operations has the following disadvantages and advantages.

Disadvantages:

1. Special efforts must be made to collect and store spores. There are also good and poor years for basidiocarp production in the field. Generally, every other year is a good year, with adequate and timely rainfall. As of this date, basidiospores are not commercially available.
2. Basidiospore collections are often contaminated with other microorganisms (yeast, bacteria, and fungi) and insects which may or may not affect health of nursery seedlings or viability of basidiospores.
3. Viability of basidiospores cannot be easily determined since they cannot be germinated consistently. The only reliable means of determining viability of basidiospores is to form ectomycorrhizae with them; this takes a minimum of three months usually.
4. Basidiospores are not as effective as vegetative inoculum in forming ectomycorrhizae on pine seedlings in nurseries. Spores take approximately 12 to 15 weeks to form detectable ectomycorrhizae, whereas vegetative inoculum takes less than 4 to 6 weeks (Marx *et al.* 1976).

5. In nurseries where recolonization of fumigated soil by naturally occurring ectomycorrhizal fungi is highly efficient, the effectiveness of basidiospores is reduced. This is not the case with vegetative inoculum following effective soil fumigation.

6. Basidiospores rarely form ectomycorrhizae on all seedlings in inoculated soil, and, with few exceptions, the overall development of ectomycorrhizae on seedlings at the end of the growing season is less than that formed by vegetative inoculum. This point is significant since increased survival and growth of seedlings in the field is correlated with the greatest amount of *Pisolithus* ectomycorrhizae (Marx *et al.* 1977).

Advantages:

1. In the South, basidiocarps can be found under pine and oak trees in great abundance on adverse sites, such as kaolin and coal spoils, especially in late summer and early fall. Several pounds of spores can be collected in only a few hours if basidiocarps are abundant. *Pisolithus* basidiocarps are easily identified since *Pisolithus* is the only fungus that produces its spores in small compartments inside the basidiocarp.

2. The spores can be extracted from the mature basidiocarps simply and economically (Marx and Bryan 1975; Marx 1976).

3. Dry spores can be stored effectively under refrigeration for at least 34 months (Marx 1976) and perhaps longer. Storage is essential since spores are normally collected in the summer or early fall and, in most instances, would not be used until the following spring.

4. Transporting basidiospores is considerably easier and more practical than transporting bulky vegetative inoculum.

5. The use of spores as inoculum for ectomycorrhizal development does not require an extended growth phase under aseptic laboratory conditions as does vegetative inoculum of *P. tinctorius* (Marx and Bryan 1975).

6. Results of this study indicate that no special equipment is needed to infest soil with basidiospores if a hydromulcher is available at the nursery.[1]

[1] This paper reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate state or federal agencies before they can be recommended.

CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife—if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

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